

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**HYPERSENSITIVE RESPONSE INDUCED RESISTANCE IN PLANTS**

This invention was made with support from the  
U.S. Government under USDA NRI Competitive Research Grant  
5 No. 91-37303-6430.

**FIELD OF THE INVENTION**

The present invention relates to imparting  
10 hypersensitive response induced resistance to plants.

**BACKGROUND OF THE INVENTION**

Living organisms have evolved a complex array  
15 of biochemical pathways that enable them to recognize and  
respond to signals from the environment. These pathways  
include receptor organs, hormones, second messengers, and  
enzymatic modifications. At present, little is known  
about the signal transduction pathways that are activated  
20 during a plant's response to attack by a pathogen,  
although this knowledge is central to an understanding of  
disease susceptibility and resistance. A common form of  
plant resistance is the restriction of pathogen  
proliferation to a small zone surrounding the site of  
25 infection. In many cases, this restriction is  
accompanied by localized death (i.e., necrosis) of host  
tissues. Together, pathogen restriction and local tissue  
necrosis characterize the hypersensitive response. In  
addition to local defense responses, many plants respond  
30 to infection by activating defenses in uninfected parts  
of the plant. As a result, the entire plant is more  
resistant to a secondary infection. This systemic  
acquired resistance can persist for several weeks or more  
(R.E.F. Matthews, Plant Virology (Academic Press, New  
35 York, ed. 2, 1981)) and often confers cross-resistance to  
unrelated pathogens (J. Kuc, in Innovative Approaches to  
Plant Disease Control, I. Chet, Ed. (Wiley, New York,  
1987), pp. 255-274, which is hereby incorporated by  
reference).

Expression of systemic acquired resistance is associated with the failure of normally virulent pathogens to ingress the immunized tissue (Kuc, J., "Induced Immunity to Plant Disease," Bioscience, 32:854-856 (1982), which is hereby incorporated by reference). Establishment of systemic acquired resistance is correlated with systemic increases in cell wall hydroxyproline levels and peroxidase activity (Smith, J.A., et al., "Comparative Study of Acidic Peroxidases Associated with Induced Resistance in Cucumber, Muskmelon and Watermelon," Physiol. Mol. Plant Pathol. 14:329-338 (1988), which is hereby incorporated by reference) and with the expression of a set of nine families of so-called systemic acquired resistance gene (Ward, E.R., et al., "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," Plant Cell 3:49-59 (1991), which is hereby incorporated by reference). Five of these defense gene families encode pathogenesis-related proteins whose physiological functions have not been established. However, some of these proteins have antifungal activity *in vitro* (Bol, J.F., et al., "Plant Pathogenesis-Related Proteins Induced by Virus Infection," Ann. Rev. Phytopathol. 28:113-38 (1990), which is hereby incorporated by reference) and the constitutive expression of a bean chitinase gene in transgenic tobacco protects against infection by the fungus *Rhizoctonia solani* (Broglie, K., et al., "Transgenic Plants with Enhanced Resistance to the Fungal Pathogen *Rhizoctonia Solani*," Science 254:1194-1197 (1991), which is hereby incorporated by reference), suggesting that these systemic acquired resistance proteins may contribute to the immunized state (Uknes, S., et al., "Acquired Resistance in *Arabidopsis*," Plant Cell 4:645-656 (1992), which is hereby incorporated by reference).

Salicylic acid appears to play a signal function in the induction of systemic acquired resistance

- 3 -

since endogenous levels increase after immunization (Malamy, J., et al., "Salicylic Acid: A Likely Endogenous Signal in the Resistance Response of Tobacco to Viral Infection," Science 250:1002-1004 (1990), which is hereby incorporated by reference) and exogenous salicylate induces systemic acquired resistance genes (Yalpani, N., et al., "Salicylic Acid is a Systemic Signal and an Inducer of Pathogenesis-Related Proteins in Virus-Infected Tobacco," Plant Cell 3:809-818 (1991), which is hereby incorporated by reference), and acquired resistance (Uknes, S., et al., "Acquired Resistance in *Arabidopsis*," Plant Cell 4:645-656 (1992), which is hereby incorporated by reference). Moreover, transgenic tobacco plants in which salicylate is destroyed by the action of a bacterial transgene encoding salicylate hydroxylase do not exhibit systemic acquired resistance (Gaffney, T., et al., "Requirement of Salicylic Acid for the Induction of Systemic Acquired Resistance," Science 261:754-296 (1993), which is hereby incorporated by reference). However, this effect may reflect inhibition of a local rather than a systemic signal function, and detailed kinetic analysis of signal transmission in cucumber suggests that salicylate may not be essential for long-distance signaling (Rasmussen, J.B., et al., "Systemic Induction of Salicylic Acid Accumulation in Cucumber after Inoculation with *Pseudomonas Syringae* pv. *Syringae*," Plant Physiol. 97:1342-1347) (1991), which is hereby incorporated by reference).

Immunization using biotic agents has been extensively studied. Green beans were systemically immunized against disease caused by cultivar-pathogenic races of *Colletotrichum lindemuthianum* by prior infection with either cultivar-nonpathogenic races (Rahe, J.E., "Induced Resistance in *Phaseolus Vulgaris* to Bean Anthracnose," Phytopathology 59:1641-5 (1969); Elliston, J., et al., "Induced Resistance to Anthracnose at a Distance from the Site of the Inducing Interaction,"

Phytopathology 61:1110-12 (1971); Skipp, R., et al., "Studies on Cross Protection in the Anthracnose Disease of Bean," Physiological Plant Pathology 3:299-313 (1973), which are hereby incorporated by reference),

5 cultivar-pathogenic races attenuated by heat in host tissue prior to symptom appearance (Rahe, J.E., et al., "Metabolic Nature of the Infection-Limiting Effect of Heat on Bean Anthracnose," Phytopathology 60:1005-9 (1970), which is hereby incorporated by reference) or

10 nonpathogens of bean. The anthracnose pathogen of cucumber, *Colletotrichum lagenarium*, was equally effective as non-pathogenic races as an inducer of systemic protection against all races of bean anthracnose. Protection was induced by *C. lagenarium* in

15 cultivars resistant to one or more races of *C. lindemuthianum* as well as in cultivars susceptible to all reported races of the fungus and which accordingly had been referred to as 'lacking genetic resistance' to the pathogen (Elliston, J., et al., "Protection of Bean

20 Against Anthracnose by *Colletotrichum* Species Nonpathogenic on Bean," Phytopathologische Zeitschrift 86:117-26 (1976); Elliston, J., et al., "A Comparative Study on the Development of Compatible, Incompatible and Induced Incompatible Interactions Between *Colletotrichum*

25 Species and *Phaseolus Vulgaris*," Phytopathologische Zeitschrift 87:289-303 (1976), which are hereby incorporated by reference). These results suggest that the same mechanisms may be induced in cultivars reported as 'possessing' or 'lacking' resistance genes (Elliston,

30 J., et al., "Relation of Phytoalexin Accumulation to Local and Systemic Protection of Bean Against Anthracnose," Phytopathologische Zeitschrift 88:114-30 (1977), which is hereby incorporated by reference). It also is apparent that cultivars susceptible to all races

35 of *C. lindemuthianum* do not lack genes for resistance mechanisms against the pathogen.

- 5 -

Kuc, J., et al., "Protection of Cucumber Against *Collectotrichum Lagenarium* by *Colletotrichum Lagenarium*," Physiological Plant Pathology 7:195-9 (1975), which is hereby incorporated by reference),  
5 showed that cucumber plants could be systemically protected against disease caused by *Colletotrichum lagenarium* by prior inoculation of the cotyledons or the first true leaf with the same fungus. Subsequently, cucumbers have been systemically protected against  
10 fungal, bacterial, and viral diseases by prior localized infection with either fungi, bacteria, or viruses (Hammerschmidt, R., et al., "Protection of Cucumbers Against *Colletotrichum Lagenarium* and *Cladosporium Cucumerinum*," Phytopathology 66:790-3 (1976); Jenns, A.  
15 E., et al., "Localized Infection with Tobacco Necrosis Virus Protects Cucumber Against *Colletotrichum Lagenarium*," Physiological Plant Pathology 11:207-12 (1977); Caruso, F.L., et al. "Induced Resistance of Cucumber to Anthracnose and Angular Leaf Spot by  
20 *Pseudomonas Lachrymans* and *Colletotrichum Lagenarium*," Physiological Plant Pathology 14:191-201 (1979); Staub, T., et al., "Systemic Protection of Cucumber Plants Against Disease Caused by *Cladosporium Cucumerinum* and *Colletotrichum Lagenarium* by Prior Localized Infection  
25 with Either Fungus," Physiological Plant Pathology, 17:389-93 (1980); Bergstrom, G.C., et al., "Effects of Local Infection of Cucumber by *Colletotrichum Lagenarium*, *Pseudomonas Lachrymans* or Tobacco Necrosis Virus on Systemic Resistance to Cucumber Mosaic Virus,"  
30 Phytopathology 72:922-6 (1982); Gessler, C., et al., "Induction of Resistance to *Fusarium* Wilt in Cucumber by Root and Foliar Pathogens," Phytopathology 72:1439-41 (1982); Basham, B., et al., "Tobacco Necrosis Virus Induces Systemic Resistance in Cucumbers Against  
35 *Sphaerotheca Fuliginea*," Physiological Plant Pathology 23:137-44 (1983), which are hereby incorporated by reference). Non-specific protection induced by infection

- 6 -

with *C. lagenarium* or tobacco necrosis virus was effective against at least 13 pathogens, including obligatory and facultative parasitic fungi, local lesion and systemic viruses, wilt fungi, and bacteria.

- 5 Similarly, protection was induced by and was also effective against root pathogens. Other cucurbits, including watermelon and muskmelon have been systemically protected against *C. lagenarium* (Caruso, F.L., et al., "Protection of Watermelon and Muskmelon Against  
10 *Colletotrichum Lagenarium* by *Colletotrichum Lagenarium*," Phytopathology 67:1285-9 (1977), which is hereby incorporated by reference).

- Systemic protection in tobacco has also been induced against a wide variety of diseases (Kuc, J., et al., "Immunization for Disease Resistance in Tobacco,"  
15 Recent Advances in Tobacco Science 9:179-213 (1983), which is hereby incorporated by reference). Necrotic lesions caused by tobacco mosaic virus enhanced resistance in the upper leaves to disease caused by the  
20 virus (Ross, A.F., et al., "Systemic Acquired Resistance Induced by Localized Virus Infections in Plants," Virology 14:340-58 (1961); Ross, A.F., et al., "Systemic Effects of Local Lesion Formation," In: Viruses of Plants pp. 127-50 (1966), which are hereby incorporated by  
25 reference). *Phytophthora parasitica* var. *nicotianae*, *P. tabacina* and *Pseudomonas tabaci* and reduced reproduction of the aphid *Myzus persicae* (McIntyre, J.L., et al., "Induction of Localized and Systemic Protection Against  
30 *Phytophthora Parasitica* var. *nicotianae* by Tobacco Mosaic Virus Infection of Tobacco Hypersensitive to the Virus," Physiological Plant Pathology 15:321-30 (1979); McIntyre, J.L., et al., "Effects of Localized Infections of  
35 *Nicotiana Tabacum* by Tobacco Mosaic Virus on Systemic Resistance Against Diverse Pathogens and an Insect," Phytopathology 71:297-301 (1981), which are hereby incorporated by reference). Infiltration of heat-killed *P. tabaci* (Lovrekovich, L., et al., "Induced Reaction

- 7 -

Against Wildfire Disease in Tobacco Leaves Treated with Heat-Killed Bacteria," Nature 205:823-4 (1965), which is hereby incorporated by reference), and *Pseudomonas solanacearum* (Sequeira, L, et al., "Interaction of Bacteria and Host Cell Walls: Its Relation to Mechanisms of Induced Resistance," Physiological Plant Pathology 10:43-50 (1977), which are hereby incorporated by reference), into tobacco leaves induced resistance against the same bacteria used for infiltration. Tobacco plants were also protected by the nematode *Pratylenchus penetrans* against *P. parasitica* var. *nicotiana* (McIntyre, J.L., et al. "Protection of Tobacco Against *Phytophthora Parasitica* Var. *Nicotianae* by Cultivar-Nonpathogenic Races, Cell-Free Sonicates and *Pratylenchus Penetrans*," Phytopathology 68:235-9 (1978), which is hereby incorporated by reference).

Cruikshank, I.A.M., et al., "The Effect of Stem Infestation of Tobacco with *Peronospora Tabacina* Adam on Foliage Reaction to Blue Mould," Journal of the Australian Institute of Agricultural Science 26:369-72 (1960), which is hereby incorporated by reference, were the first to report immunization of tobacco foliage against blue mould (i.e., *P. tabacina*) by stem injection with the fungus, which also involved dwarfing and premature senescence. It was recently discovered that injection external to the xylem not only alleviated stunting but also promoted growth and development. Immunized tobacco plants, in both glasshouse and field experiments, were approximately 40% taller, had a 40% increase in dry weight, 30% increase in fresh weight, and 4-6 more leaves than control plants (Tuzun, S., et al., "The Effect of Stem Injections with *Peronospora Tabacina* and Metalaxyl Treatment on Growth of Tobacco and Protection Against Blue Mould in the Field," Phytopathology 74:804 (1984), which is hereby incorporated by reference). These plants flowered approximately 2-3 weeks earlier than control plants



(Tuzun, S., et al., "Movement of a Factor in Tobacco Infected with *Peronospora Tabacina* Adam which Systemically Protects Against Blue Mould," Physiological Plant Pathology 26:321-30 (1985), which is hereby  
5 incorporated by reference).

Systemic protection does not confer absolute immunity against infection, but reduces the severity of the disease and delays symptom development. Lesion number, lesion size, and extent of sporulation of fungal  
10 pathogens are all decreased. The diseased area may be reduced by more than 90%.

When cucumbers were given a 'booster' inoculation 3-6 weeks after the initial inoculation, immunization induced by *C. lagenarium* lasted through  
15 flowering and fruiting (Kuc, J., et al., "Aspects of the Protection of Cucumber Against *Colletotrichum Lagenarium* by *Colletotrichum Lagenarium*," Phytopathology 67:533-6 (1977), which is hereby incorporated by reference). Protection could not be induced once plants had set  
20 fruit. Tobacco plants were immunized for the growing season by stem injection with sporangia of *P. tabacina*. However, to prevent systemic blue mould development, this technique was only effective when the plants were above  
20 cm in height.

25 Removal of the inducer leaf from immunized cucumber plants did not reduce the level of immunization of pre-existing expanded leaves. However, leaves which subsequently emerged from the apical bud were progressively less protected than their predecessors  
30 (Dean, R.A., et al., "Induced Systemic Protection in Cucumber: Time of Production and Movement of the 'Signal'," Phytopathology 76:966-70 (1986), which is hereby incorporated by reference). Similar results were reported by Ross, A.F., "Systemic Effects of Local Lesion  
35 Formation," In: Viruses of Plants pp. 127-50 (1966), which is hereby incorporated by reference, with tobacco (local lesion host) immunized against tobacco mosaic

- 9 -

virus by prior infection with tobacco mosaic virus. In contrast, new leaves which emerged from scions excised from tobacco plants immunized by stem-injection with *P. tabacina* were highly protected (Tuzun, S., et al.,  
5 "Transfer of Induced Resistance in Tobacco to Blue Mould (Peronospora Tabacina Adam.) Via Callus," Phytopathology 75:1304 (1985), which is hereby incorporated by reference). Plants regenerated via tissue culture from leaves of immunized plants showed a significant reduction  
10 in blue mould compared to plants regenerated from leaves of non-immunized parents. Young regenerants only showed reduced sporulation. As plants aged, both lesion development and sporulation were reduced. Other investigators, however, did not reach the same  
15 conclusion, although a significant reduction in sporulation in one experiment was reported (Lucas, J.A., et al., "Nontransmissibility to Regenerants from Protected Tobacco Explants of Induced Resistance to *Peronospora Hyoscyami*," Phytopathology 75:1222-5 (1985),  
20 which is hereby incorporated by reference).

Protection of cucumber and watermelon is effective in the glasshouse and in the field (Caruso, F.L., et al., "Field Protection of Cucumber Against *Colletotrichum Lagenarium* by *C. Lagenarium*,"  
25 Phytopathology 67:1290-2 (1977), which is hereby incorporated by reference). In one trial, the total lesion area of *C. lagenarium* on protected cucumber was less than 2% of the lesion areas on unprotected control plants. Similarly, only 1 of 66 protected, challenged  
30 plants died, whereas 47 of 69 unprotected, challenged watermelons died. In extensive field trials in Kentucky and Puerto Rico, stem injection of tobacco with sporangia of *P. tabacina* was at least as effective in controlling blue mould as the best fungicide, metalaxyl. Plants were  
35 protected 95-99%, based on the necrotic area and degree of sporulation, leading to a yield increase of 10-25% in cured tobacco.

- 10 -

Induced resistance against bacteria and viruses appears to be expressed as suppression of disease symptoms or pathogen multiplication or both (Caruso, F.L., et al., "Induced Resistance of Cucumber to Anthracnose and Angular Leaf Spot by *Pseudomonas Lachrymans* and *Colletotrichum Lagenarium*," Physiological Plant Pathology 14:191-201 (1979); Doss, M., et al., "Systemic Acquired Resistance of Cucumber to *Pseudomonas Lachrymans* as Expressed in Suppression of Symptoms, but not in Multiplication of Bacteria," Acta Phytopathologia Academiae Scientiarum Hungaricae 16:(3-4), 269-72 (1981); Jenns, A.E., et al., "Non-Specific Resistance to Pathogens Induced Systemically by Local Infection of Cucumber with Tobacco Necrosis Virus, *Colletotrichum Lagenarium* or *Pseudomonas Lachrymans*," Phytopathologia Mediterranea 18:129-34 (1979), which are hereby incorporated by reference).

As described above, research concerning systemic acquired resistance involves infecting plants with infectious pathogens. Although studies in this area are useful in understanding how systemic acquired resistance works, eliciting such resistance with infectious agents is not commercially useful, because such plant-pathogen contact can weaken or kill plants. The present invention is directed to overcoming this deficiency.

#### SUMMARY OF THE INVENTION

The present invention relates to a method of imparting pathogen resistance to plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant under conditions where the polypeptide or protein contacts cells of the plant.

Another aspect of the present invention relates to a pathogen-resistant plant with cells in contact with

- 11 -

non-infectious hypersensitive response elicitor polypeptide or protein.

Yet another aspect of the present invention relates to a composition for imparting pathogen resistance to plants. The composition includes a non-infectious, hypersensitive response elicitor polypeptide or protein and a carrier.

The present invention has the potential to: treat plant diseases which were previously untreatable; treat diseases systemically that one would not want to treat separately due to cost; and avoid the use of infectious agents to treat diseases. The present invention can impart resistance without using agents pathogenic to the plants being treated or to plants situated nearby those treated. Since the present invention involves use of a natural product that is fully biodegradable, the environment would not be contaminated.

#### BRIEF DESCRIPTION OF THE DRAWINGS

20

Figure 1 shows the genetic organization of the gene cluster encoding the hypersensitive response elicitor polypeptide or protein for *Erwinia amylovora* (i.e. *hrpN*). The top line shows the restriction enzyme map of plasmid vector pCPP430, where E=Eco RI, B=Bam HI, and H=Hind III. The rectangles represent transcriptional units, and the arrows under the rectangles indicate the directions of transcription. The bigger arrow indicates the region necessary for ultimate translation of the hypersensitive response elicitor polypeptide or protein. pCPP430 *hrpN* is the derivative of pCPP430 in which *hrpN* is mutated by the insertion of transposor TnStac.

Figure 2 is a map of plasmid vector pCPP9. Significant features are the mobilization (mob) site for conjugation; the cohesive site of  $\lambda$  (cos); and the partition region (par) for stable inheritance of the plasmid. B, BamHI; E, EcoRI; H, HindIII; P, PstI; S,

- 12 -

SaII; Sm, SmaI; oriV, origin of replication; Sp<sup>r</sup>, spectinomycin resistance; Sm<sup>r</sup>, streptomycin resistance.

#### DETAILED DESCRIPTION OF THE INVENTION

5

The present invention relates to a method of imparting pathogen resistance to plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or  
10 part of a plant under conditions where the polypeptide or protein contacts all or part of the cells of the plant.

Another aspect of the present invention relates to a pathogen-resistant plant with cells in contact with a non-infectious hypersensitive response elicitor  
15 polypeptide or protein.

Yet another aspect of the present invention relates to a composition for imparting pathogen resistance to plants. The composition includes a non-infectious hypersensitive response elicitor polypeptide  
20 or protein and a carrier.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention can correspond to hypersensitive response elicitor polypeptides or proteins derived from a wide variety of  
25 pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Preferred pathogens include *Erwinia amylovora*, *Erwinia chrysanthemi*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, or mixtures thereof.

30 For purposes of the present invention, non-infectious forms of the hypersensitive response elicitor polypeptide or protein can induce a hypersensitive response without causing disease in the plant with which the polypeptide or protein is contacted. This can be  
35 achieved in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and

- 13 -

are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and  
5 naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

In one embodiment of the present invention, the hypersensitive response elicitor polypeptides or proteins can be isolated from their corresponding  
10 organisms and applied to plants. Such isolation procedures are well known, as described in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia  
15 Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543 - 553 (1994); He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin<sub>pgs</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in  
20 Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby  
25 incorporated by reference. See also pending U.S. Patent Application Serial Nos. 08/200,024 and 08/062,024, which are hereby incorporated by reference. Preferably, however, the isolated hypersensitive response elicitor polypeptides or proteins of the present invention are  
30 produced recombinantly and purified as described below.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants by applying bacteria containing genes encoding the  
35 hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can

- 14 -

contact plant cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria in planta or just prior to introduction of the bacteria to the plants.

5 In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example,  
 10 *E. coli*, which do not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species (other than *E. coli*) can also be used in this embodiment of the  
 15 present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein.  
 20 Examples of such bacteria are noted above. However, in this embodiment these bacteria are applied to plants which are not susceptible to the disease carried by the bacteria. For example, *Erwinia amylovora* causes disease in apple or pear but not in tomato. However, such  
 25 bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato to impart pathogen resistance without causing disease in that species.

30 The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

35 Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
 1 5 10 15  
 Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
 20 25 30  
 40

- 15 -

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr  
                   35                                  40                                  45  
 Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu  
 5                  50                                  55                                  60  
 Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser  
                   65                                  70                                  75                                  80  
 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
 10                                  85                                  90                                  95  
 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp  
                   100                                  105                                  110  
 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln  
                   115                                  120                                  125  
 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met  
 20                  130                                  135                                  140  
 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly  
                   145                                  150                                  155                                  160  
 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly  
 25                                  165                                  170                                  175  
 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu  
                   180                                  185                                  190  
 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala  
                   195                                  200                                  205  
 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val  
 35                  210                                  215                                  220  
 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
                   225                                  230                                  235                                  240  
 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
                   245                                  250                                  255  
 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
                   260                                  265                                  270  
 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
                   275                                  280                                  285  
 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
 50                  290                                  295                                  300  
 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
                   305                                  310                                  315                                  320  
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
                   325                                  330                                  335  
 Asn Ala

60.

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi*



hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

5	CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CCGTATTCTGA CACCGTTACG	60
	GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCCGCCGA ATCCGGCGTC	120
	GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG	180
10	CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG	240
	TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
15	CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACCT GGCGGGAATG	360
	ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC	420
	CGATCATTAA GATAAAGGCG GCTTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
20	CACCGTCGGC GTCACCTCAGT AACAAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG	540
	GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
25	AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC	660
	TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT	720
	GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT	780
30	GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
	TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA	900
35	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
	CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG	1080
40	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200

- 17 -

GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260  
 CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320  
 5 TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380  
 GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440  
 CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500  
 10 TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560  
 GGCTGTCGTC GGCATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620  
 15 ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680  
 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740  
 ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800  
 20 GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860  
 CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG 1920  
 25 CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980  
 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040  
 AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100  
 30 GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

The hypersensitive response elicitor polypeptide or  
 protein derived from *Erwinia amylovora* has an amino acid  
 35 sequence corresponding to SEQ. ID. No. 3 as follows:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15  
 40 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30  
 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45  
 45 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60

- 18 -

	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu	65	70	75	80
5	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu	85	90	95	
	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr	100	105	110	
10	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro	115	120	125	
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser	130	135	140	
15	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln	145	150	155	160
	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly	165	170	175	
20	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu	180	185	190	
25	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly	195	200	205	
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly	210	215	220	
30	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu	225	230	235	240
	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln	245	250	255	
35	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln	260	265	270	
40	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe	275	280	285	
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	290	295	300	
45	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro	305	310	315	320
	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser	325	330	335	
50	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn	340	345	350	
55	Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn	355	360	365	
	Gly	Asn	Leu	Gln	His	Ala	Val	Pro	Val	Val	Leu	Arg	Trp	Val	Leu	Met	370	375	380	
60	Pro																385			

This hypersensitive response elicitor polypeptide or protein  
 65 has a molecular weight of about 37 kDa, it has a pI of

- 19 -

approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

15	ATGAGTCTGA ATACAAGTGG GCTGGGAGCG TCAACGATGC AAATTTCTAT CGGCGGTGCG	60
	GGCGGAAATA ACGGGTTGCT GGGTACCAGT CGCCAGAATG CTGGGTTGGG TGGCAATTCT	120
	GCACTGGGGC TGGGCGGCGG TAATCAAAAT GATACCGTCA ATCAGCTGGC TGGCTTACTC	180
20	ACCGGCATGA TGATGATGAT GAGCATGATG GCGGTTGGTG GGCTGATGGG CGGTGGCTTA	240
	GGCGGTGGCT TAGGTAATGG CTTGGGTGGC TCAGGTGGCC TGGGCGAAGG ACTGTGGAAC	300
	GCGCTGAACG ATATGTTAGG CGGTTTCGCTG AACACGCTGG GCTCGAAAGG CGGCAACAAT	360
25	ACCACTTCAA CAACAAATTC CCCGCTGGAC CAGGCGCTGG GTATTAACTC AACGTCCCAA	420
	AACGACGATT CCACCTCCGG CACAGATTCC ACCTCAGACT CCAGCGACCC GATGCAGCAG	480
30	CTGCTGAAGA TGTTCAAGCA GATAATGCAA AGCCTGTTTG GTGATGGGCA AGATGGCACC	540
	CAGGGCAGTT CCTCTGGGGG CAAGCAGCCG ACCGAAGGCG AGCAGAACGC CTATAAAAAA	600
	GGAGTCACTG ATGCGCTGTC GGGCCTGATG GGTAATGGTC TGAGCCAGCT CCTTGGCAAC	660
35	GGGGGACTGG GAGGTGGTCA GGGCGGTAAT GCTGGCACGG GTCTTGACGG TTCGTCGCTG	720
	GGCGGCAAAG GGCTGCAAAA CCTGAGCGGG CCGGTGGACT ACCAGCAGTT AGGTAACGCC	780
40	GTGGGTACCG GTATCGGTAT GAAAGCGGGC ATTCAGGCGC TGAATGATAT CGGTACGCAC	840
	AGGCACAGTT CAACCCGTTT TTTGTCATAT AAAGGCGATC GGGCGATGGC GAAGGAAATC	900
	GGTCAGTTCA TGGACCAGTA TCCTGAGGTG TTTGGCAAGC CGCAGTACCA GAAAGGCCCG	960
45	GGTCAGGAGG TGAAAACCGA TGACAAATCA TGGGCAAAAG CACTGAGCAA GCCAGATGAC	1020
	GACGGAATGA CACCAGCCAG TATGGAGCAG TTCAACAAAG CCAAGGGCAT GATCAAAAGG	1080
50	CCCATGGCGG GTGATACCGG CAACGGCAAC CTGCAGCACG CCGTGCCGGT GGTTCCTCGC	1140
	TGGGTATTGA TGCCATGA	1158

- 20 -

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

5	Met	Gln	Ser	Leu	Ser	Leu	Asn	Ser	Ser	Ser	Leu	Gln	Thr	Pro	Ala	Met	1	5	10	15
10	Ala	Leu	Val	Leu	Val	Arg	Pro	Glu	Ala	Glu	Thr	Thr	Gly	Ser	Thr	Ser	20	25	30	
	Ser	Lys	Ala	Leu	Gln	Glu	Val	Val	Val	Lys	Leu	Ala	Glu	Glu	Leu	Met	35	40	45	
15	Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala	50	55	60	
	Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val	65	70	75	
20	Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe	85	90	95	
25	Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met	100	105	110	
	Thr	Gln	Val	Leu	Asn	Gly	Leu	Ala	Lys	Ser	Met	Leu	Asp	Asp	Leu	Leu	115	120	125	
30	Thr	Lys	Gln	Asp	Gly	Gly	Thr	Ser	Phe	Ser	Glu	Asp	Asp	Met	Pro	Met	130	135	140	
	Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro	145	150	155	
35	Lys	Pro	Asp	Ser	Gly	Ser	Trp	Val	Asn	Glu	Leu	Lys	Glu	Asp	Asn	Phe	165	170	175	
40	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile	180	185	190	
	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly	195	200	205	
45	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser	210	215	220	
	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	225	230	235	
50	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	245	250	255	
55	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val	260	265	270	
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln	275	280	285	
60	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala	290	295	300	
	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala				

	ATGCAGAGTC	TCAGTCTTAA	CAGCAGCTCG	CTGCAAAACCC	CGGCAATGGC	CCTTGTCCTG	60
25	GTACGTCCTG	AAGCCGAGAC	GA CTGGCAGT	ACGTCGAGCA	AGGCGCTTCA	GGAAAGTTGTC	120
	GTGAAGCTGG	CCGAGGAACT	GATGCGCAAT	GGTCAACTCG	ACGACAGCTC	GCCATTGGGA	180
	AAACTGTTGG	CCAAGTCGAT	GGCCGCAGAT	GGCAAGGCGG	GCGGCGGTAT	TGAGGATGTC	240
30	ATCGCTGCGC	TGGACAAGCT	GATCCATGAA	AAGCTCGGTG	ACAAC TTCGG	CGCGTCTGCG	300
	GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
35	AAGTCGATGC	TCGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
	GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCCG	ACAGTTTCCC	480
	AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAAC TTCCT	TGATGGCGAC	540
40	GAAACGGCTG	CGTTCCGTTT	GGCACTCGAC	ATCATTGGCC	AGCAACTGGG	TAATCAGCAG	600
	AGTGACGCTG	GCAGTCTGGC	AGGGACGGGT	GGAGGTCTGG	GCACTCCGAG	CAGTTTTTCC	660
45	AACAAC TCGT	CCGTGATGGG	TGATCCGCTG	ATCGACGCCA	ATACCGGTCC	CGGTGACAGC	720
	GGCAATACCC	GTGGTGAAGC	GGGGCAACTG	ATCGGCGAGC	TTATCGACCG	TGGCCTGCAA	780
	TCGGTATTGG	CCGGTGGTGG	ACTGGGCACA	CCCGTAAACA	CCCCGCAGAC	CGGTACGTCG	840
50	GCGAATGGCG	GACAGTCCGC	TCAGGATCTT	GATCAGTTGC	TGGGCGGCTT	GCTGCTCAAG	900
	GGCCTGGAGG	CAACGCTCAA	GGATGCCGGG	CAAACAGGCA	CCGACGTGCA	GTCGAGCGCT	960
55	GCGCAAAATCG	CCACCTTGCT	GGTCAGTACG	CTGCTGCAAG	GCACCCGCAA	TCAGGCTGCA	1020

GCCTGA

1026

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

10	Met	Ser	Val	Gly	Asn	Ile	Gln	Ser	Pro	Ser	Asn	Leu	Pro	Gly	Leu	Gln	1	5	10	15
	Asn	Leu	Asn	Leu	Asn	Thr	Asn	Thr	Asn	Ser	Gln	Gln	Ser	Gly	Gln	Ser	20	25	30	
15	Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile	35	40	45	
	Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly	50	55	60	
20	Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala	65	70	75	80
	Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser	85	90	95	
25	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	100	105	110	
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	115	120	125	
30	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	130	135	140	
35	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	145	150	155	160
	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	165	170	175	
40	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	180	185	190	
45	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	195	200	205	
	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	210	215	220	
50	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp	225	230	235	240
	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn	245	250	255	
55	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln	260	265	270	
60	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly	275	280	285	
	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser	290	295	300	

- 23 -

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
305 310 315 320

5 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
325 330 335

Gln Ser Thr Ser Thr Gln Pro Met  
340

10

It is encoded by a DNA molecule having a nucleotide  
sequence corresponding SEQ. ID. No. 8 as follows:

15 ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60  
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120  
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180  
20 GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240  
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300  
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA 360  
25 GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420  
GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480  
30 GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540  
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600  
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660  
35 GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720  
CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG 780  
40 ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840  
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900  
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960  
45 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020  
ACGCAGCCGA TGTA 1035

50

Further information regarding the hypersensitive response  
elicitor polypeptide or protein derived from *Pseudomonas*  
*solanacearum* is set forth in Arlat, M., F. Van Gijsegem,  
J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a  
55 Protein which Induces a Hypersensitive-like Response in  
Specific Petunia Genotypes, is Secreted via the Hrp  
Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533  
(1994), which is hereby incorporated by reference.



The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

5 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala  
1 5 10 15  
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
10 20 25

This sequence is an amino terminal sequence having 26 residues only from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *xanthomonas campestris* pathovars.

The above elicitors are exemplary. Other elicitors can be identified by growing bacteria that elicit a hypersensitive response under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

25           It is also possible to use fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens, in the method of the present invention.

30           Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or  
35 *in vivo* in bacterial cells to yield a smaller protein or a peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length  
40 elicitor protein with proteolytic enzymes like

- 25 -

chymotrypsin or Staphylococcus proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for increase and expression of a truncated peptide or protein,

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide.

The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant *E. coli*. To isolate the protein, the *E. coli* host cell carrying a recombinant plasmid is propagated, homogenized, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the polypeptide or protein of the present invention is subjected to gel

- 26 -

filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

5           The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA  
10 molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation  
15 of the inserted protein-coding sequences.

          U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage  
20 and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

          Recombinant genes may also be introduced into  
25 viruses, such as vaccina virus. Recombinant viruses can be generated by transection of plasmids into cells infected with virus.

          Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector  
30 system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif,  
35 which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes,"

- 27 -

Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1982), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further,

- 28 -

procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the

- 29 -

promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the  
5 addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in  
10 procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any  
15 combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a Shine-Dalgarno (SD) sequence about 7-9 bases 5' to the initiation codon (ATG) to provide a ribosome binding site. Thus, any SD-ATG  
20 combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-  
25 ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein  
30 has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to,  
35 bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

- 30 -

The method of the present invention can be utilized to treat a wide variety of plants to impart pathogen resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi.

Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: Tobacco mosaic virus and tomato mosaic virus.

Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with the present invention: *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*.

Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

The method of the present invention can be carried out through a variety of procedures for applying the hypersensitive response elicitor polypeptide or protein to all or part of the plant being treated. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include

- 31 -

high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. Other suitable application procedures can be envisioned by those skilled in the art provided they are  
5 able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant.

The hypersensitive response elicitor polypeptide or protein can be applied to plants in accordance with the present invention alone or in a  
10 mixture with other materials.

One aspect of the present invention involves a composition for imparting pathogen resistance to plants containing a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water  
15 or aqueous solutions. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, and mixtures thereof. Suitable  
20 fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering  
25 agents, wetting agents, and abrading agents. These materials can be used to facilitate the process of the present invention.

#### EXAMPLES

30

**Example 1 - Harpin-induced Resistance of Tomato Against the Southern Bacterial Wilt Disease (*Pseudomonas solanacearum*)**

35

Two-week-old tomato seedlings, grown in 8 x 15 cm flats in the greenhouse were treated as follows: 20 plants were used for each of the six treatments, which



- 32 -

were designated A through F, and are described as follows:

5 (A) About 100  $\mu$ l of a 200  $\mu$ g/ml crude harpin (i.e. hypersensitive response elicitor polypeptide or protein) preparation (Z-M. Wei, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference). was infiltrated into the lowest true leaf of each of the seedlings.

10 (B) The same harpin preparation as used in (A) was sprayed with 400-mesh carborundum onto the leaf surface of the seedlings and then gently rubbed in with the thumb.

15 (C) *E. coli* DH5(pCPP430) (See Figure 1 for map of plasmid vector pCPP430) was grown in LB medium to  $OD_{620}=0.7$ . The culture was centrifuged and then resuspended in 5 mM of potassium phosphate buffer pH 6.5. About 100  $\mu$ l of cell suspension was infiltrated into each leaf of the seedlings.

20 (D) *E. coli* DH5(pCPP430::hrpN) (See Figure 1 for map of plasmid vector pCPP430::hrpN) was used as in (C). The cells were grown, and the suspension and the amount of inoculum used were the same as described in (C).

25 (E) For *E. coli* DH5(pCPP9) (See Figure 2), the cells were grown and the suspension and the amount of inoculum used were the same as described in (C).

(F) Infiltration of leaves with 5mM potassium phosphate buffer was as described in (C).

30 The challenge pathogenic bacterium, *Pseudomonas solanacearum* strain K60, was grown in King's medium B to  $OD_{620}=0.7$  (about  $10^8$  cfu/ml). The culture was centrifuged and resuspended in 100 volume of 5 mM potassium phosphate buffer to a final concentration of about  $1 \times 10^6$  cfu/ml.

35 Three days after the tomato seedlings were treated with harpin or bacteria, they were pulled up and about one cm of roots were cut off with scissors. The

- 33 -

seedlings were then dipped into the suspension of *P. solanacearum* K60 for 3 min. The inoculated plants were replanted into the same pots. The plants were left in a greenhouse, and the disease incidence was recorded 7 days after inoculation.

A. Effect of treatment with harpin

After 24 hours, only those leaf portions that had been infiltrated with harpin or *E. coli* DH5(pCPP430) had collapsed. Leaves sprayed with harpin and carborundum showed only spotty necrosis.

B. Effect of treatment with harpin on the development of Southern Bacterial Wilt.

None of the 20 harpin-infiltrated plants showed any symptoms one week after inoculation with *P. solanacearum* K60 (Table 1). One out of the 20 plants showed stunting symptoms. However, 7 of the 20 buffer-infiltrated plants showed stunting symptoms. Treatment with *E. coli* DH5(pCPP430) (a transposon-induced mutant unable to elicit the hypersensitive collapse) or *E. coli* DH5(pCPP9) did not show significant difference compared to the plants treated with buffer. These results suggest that harpin or *E. coli* DH5(pCPP430), which produces harpin, induced resistance in the tomato plants to southern bacterial wilt caused by *P. solanacearum* K60.

- 34 -

Table 1. Disease incidence of tomato seedlings 7 and 14 days after inoculation with *P. solanacearum* K60.

		Number of Plants			
		Day 7		Day 14	
	<u>Treatment</u>	<u>Stunted</u>	<u>Healthy</u>	<u>Stunted</u>	<u>Healthy</u>
5	A. Harpin infiltration	0	20	2	18
10	B. Harpin spray	1	19	3	17
	C. <i>E. coli</i> DH5 (pCPP430)	2	18	3	17
	D. <i>E. coli</i> DH5 (pCPP430 <sup>-</sup> )	4	16	7	13
	E. <i>E. coli</i> DH5 (pCPP9)	5	15	6+1 wilted	13
	F. Buffer	7	13	8+1 wilted	11
15	No pathogen	0	20	0	20

Four weeks after inoculation, plants treated with the harpin or *E. coli* DH5 (pCPP430) were taller and broader as compared to those treated with buffer. The average heights of 10 plants that had been infiltrated with harpin or buffer are given in Table 2.

Table 2. Heights (cm) of tomato plants four weeks after inoculation with *Pseudomonas solanacearum* K60, following treatment with harpin or buffer.

	<u>Infiltrated with Buffer Not inoculated</u>	<u>Infiltrated with Harpin Inoculated with K60</u>	<u>Infiltrated with Buffer Inoculated with K60</u>
30	36	32	11
	41	29	21
	35	38	33
	34	35	12
35	39	37	15
	35	33	32
	36	22	25
	35	35	15
	41	40	37
40	37	29	38
	Average 36.9	33	23.9

- 35 -

**Example 2 - Harpin-induced Resistance of Tomato against Southern Bacterial Wilt Disease *Pseudomonas solanacearum***

5

All the methods used for infiltration and inoculation were the same as described in Example 1, except that the concentration of *P. solanacearum* K60 was about  $5 \times 10^4$  cfu/ml.

10 The buffer-infiltrated plants showed symptoms 15 days after inoculation with *P. solanacearum* K60. Six out of 20 plants showed stunting symptoms after 15 days; 2 plants were wilted after 21 days. The wilted plants eventually died. However, none of the 20 harpin-treated  
15 plants showed stunting symptoms. Three weeks after inoculation, 3 of the 20 harpin-treated plants showed stunting symptoms. It is possible that after three weeks, the plants may have lost their induced resistance. As in the first experiment, the overall girth and heights  
20 of the harpin-treated plants were greater than those treated with buffer.

**Example 3 - Harpin-induced Resistance of Tomato against Southern Bacterial Wilt Disease *Pseudomonas solanacearum***

25

This experiment was similar to Example 1, except that additional inoculum of *Pseudomonas solanacearum* K60 was added to the pots containing the  
30 treated tomato plants.

Harpin was infiltrated into two-week-old tomato seedlings. Two panels of each plant were infiltrated with about 200  $\mu$ l harpin suspended in 5 mM of potassium  
35 phosphate buffer at the concentration about 200  $\mu$ g/ml. A total of 20 tomato seedlings were infiltrated. The same number of tomato seedlings were infiltrated with buffer. After two days, the plants were inoculated with *Pseudomonas solanacearum* K60 by root-dipping. The  
40 harpin- or buffer-infiltrated plants were pulled from the soil mix and small amounts of roots were cut off with scissors and then the remaining roots were dipped into a

- 36 -

suspension of *P. solanacearum* K60 for three minutes. The concentration of the bacterial cell suspension was about  $5 \times 10^8$  cfu/ml. The seedlings were replanted into the same pot. An additional 3 ml of bacterial suspension was  
 5 added to the soil of each individual 4-inch diameter pot. Disease incidence was scored after one week. All the experiments were done in the greenhouse with limited temperature control.

After three weeks, 11 of the 20 buffer-  
 10 infiltrated tomato plants had died and 2 plants that had wilted recovered, but remained severely stunted. Only 4 plants grew normally compared with non-inoculated tomatoes. However, 15 of the harpited plants appeared  
 15 healthy; three plants were stunted and two plants were wilted 3 weeks after inoculation. These results are summarized below in Table 3.

Table 3. Harpin-induced resistance of tomato against  
 20 bacterial wilt disease caused by *P. solanacearum*

		<u>Weeks After Inoculation</u>		
<u>Treatment</u>		<u>1</u>	<u>2</u>	<u>3</u>
25	Harpin			
	Healthy	20	17	15
	Wilted	0	1	2
	Stunted	0	2	3
30	Buffer			
	Healthy	8	5	4
	Wilted	8	12	13
	Stunted	4	3	3

35 Example 4 - Harpin-induced Resistance of Tobacco to Tobacco Mosaic Virus

One panel of a lower leaf of four-week old  
 40 tobacco seedlings (cultivar, Xanthi, with N gene) were infiltrated with *E. amylovora* harpin at the concentration

- 37 -

of 200  $\mu\text{g/ml}$ . After three days, the plants were challenged with tobacco mosaic virus ("TMV"). Two concentrations of the virus (5  $\mu\text{g}$  and 100  $\mu\text{g/ml}$ ) were used. About 50  $\mu\text{l}$  of the virus suspension was deposited on one upper tobacco leaf. The leaf was dusted with 400-mesh carborundum and the leaves gently rubbed. Each concentration was tested on three plants. Necrotic lesions were counted 4 days after inoculation and on two subsequent days and the mean number on three leaves is reported (Table 4). It was difficult to distinguish the individual lesions by Day 10 because some of the necrotic lesions had merged together. Therefore, the number of lesions recorded seemed less than those recorded on Day 7. The size of the necrotic lesions in buffer-treated leaves was much larger than the harpin-treated leaves.

Table 4. Harpin-induced resistance of tobacco against TMV from inoculation with 5  $\mu\text{g/ml}$  of virus

<u>Mean Number of Lesions/Leaf</u>			
<u>Treatment</u>	<u>Day 4</u>	<u>Day 7</u>	<u>Day 10</u>
Harpin	21	32	35
Buffer	67	102	76

There was no significant difference in the number of local lesions that developed on the harpin-treated and buffer-treated tobacco when the tobacco mosaic virus inoculum concentration was 100  $\mu\text{g/ml}$ .

**Example 5 - Harpin-induced Resistance of Tomato to Fusarium Wilt Disease**

Six-week-old tomato plants were treated with harpin as described for Example 3. The fungal pathogen, *Fusarium oxysporum*, was grown on Lima Bean Agar medium for 5 days at 27°C. Two entire agar plates with mycelia were blended for 2 minutes in 20 ml of 5 mM potassium phosphate buffer. The roots of harpin- or buffer-treated tomato plants were wounded by plunging a wooden stake

- 38 -

into the soil of the pots. Then, 3 ml of the fungal suspension was poured into the soil of each 4-inch pot. The inoculated plants remained in a controlled environment chamber at 24°C with 16 hours of light per day. Disease incidence was recorded 7 days after inoculation. Each treatment was applied to 10 plants. The results are shown below in Table 5.

Table 5. Effect of harpin or buffer treatment on Fusarium wilt disease of tomato

Number of plants (of 10) showing wilt symptoms at the indicated time post-inoculation

Treatment	Day 7	Day 10	Day 15	Day 20
Harpin	1	2	4	4 (1 dead)
Buffer	3	6	7	7 (4 dead)

Example 6 - Harpin-Induced Resistance of Tobacco Against Wildfire Disease (*Pseudomonas syringae* pv. *tabaci*).

Harpin was infiltrated into single panels of the lower leaves of 4-week-old tobacco plants (20 cm high). After three days, suspensions of *Pseudomonas syringae* pv. *tabaci* were infiltrated into single panels of upper leaves. Four days later, disease incidence was recorded, as set forth in Table 6.

Table 6. Symptoms of infection by Wildfire disease in tobacco leaves inoculated with *Pseudomonas syringae* pv. *tabaci* following treatment of lower leaves with harpin.

<u>Concentration of P.s. tabaci</u>	<u>Treated with Harpin</u>	<u>Not treated with Harpin</u>
10 <sup>4</sup> cfu/ml	no symptoms	necrosis and water-soaking
10 <sup>5</sup> cfu/ml	no symptoms	necrosis and water-soaking
10 <sup>6</sup> cfu/ml	no symptoms	necrosis and water-soaking
10 <sup>7</sup> cfu/ml	no symptoms	necrosis and water-soaking
10 <sup>8</sup> cfu/ml	necrosis	necrosis and water-soaking

**Example 7 - Harpin-induced Resistance of Geranium  
(*Pelargonium hortorum*) Against Bacterial  
Leaf Spot (*Xanthomonas campestris* pv.  
*pelargonii*)**

5

This experiment was done with rooted cuttings of geranium growing in individual 4" or 6" pots in an artificial soil mix in a greenhouse. Two lower leaves on each plant were infiltrated with either 0.05 M potassium phosphate buffer, pH 6.5 (control), or harpin or a suspension of *Escherichia coli* DH5(pCPP430) (the entire cloned hrp gene cluster of *E. amylovora*). Two to seven days following infiltration, all the plants were inoculated with a pure culture of the bacterial leaf spot pathogen, *Xanthomonas campestris* pv. *pelargonii*. A suspension of the bacteria ( $5 \times 10^6$  cfu/ml) was atomized over both upper and lower leaf surfaces of the plants at low pressure. Each treatment was applied to two plants (designated "A" and "B" in Table 7). The plants were maintained in a closed chamber for 48 hours with supplemental misting supplied by cool-mist foggers. Then, the plants were maintained on the greenhouse bench subject to ambient humidity and temperature of 23°C to 32°C for 10 days before disease development was assessed.

25

Table 7. Effect of harpin and the hrp gene cluster of *Erwinia amylovora* on the development of bacterial leaf spot of geranium.

30

Time between treatment and inoculation with  
*Xanthomonas campestris* pv. *pelargonii*

35

Treatment	7 Days		5 Days		4 Days		3 Days		2 days	
	<u>Plant</u>		<u>Plant</u>		<u>Plant</u>		<u>Plant</u>		<u>Plant</u>	
	A	B	A	B	A	B	A	B	A	B
Buffer	3*	5	5	4	3	2	4	3	4	5
Harpin	0	0	0	0	0	0	1	0	0	0
DH5 (pCPP430)	0	0	NT	NT	0	0	0	1	1	0

40

45



- 5 \* Numbers in table are the number of leaves showing disease symptoms (pronounced necrosis, chlorosis, or wilting) 10 days following inoculation.

10 **Example 8 - Activity of several harpins in inducing resistance to Wildfire Disease caused by *Pseudomonas syringae* pv. *tabaci***

15 Tobacco plants (*Nicotiana tabacum* var. *Xanthi*) were grown in the greenhouse. At 4 weeks of age, harpin preparations were infiltrated into a single panel of two lower leaves of each plant. Twelve plants were treated with each harpin preparation, and three were treated with the same potassium phosphate buffer that was used to  
20 prepare the harpins. The hypersensitive necrosis developed within 24 hours in the panels of the leaves infiltrated with the harpin preparations, but not with buffer.

25 At 7, 10, 11, and 12 days after harpin treatment, all plants were inoculated with suspensions of  $10^4$  to  $10^6$  cells/ml of *Pseudomonas syringae* pv. *tabaci* by infiltrating panels on upper leaves. Plants were incubated in the greenhouse for 7 days before disease development was evaluated. The results are tabulated as  
30 follows in Table 8:

**Table 8**

<u>Harpin source</u>		<u>Days between treatment and inoculation</u>											
		<u>12</u>			<u>11</u>			<u>10</u>			<u>7</u>		
	log [Inoc.]	4	5	6	4	5	6	4	5	6	4	5	6
35	None (buffer)	+	+	+	+	+	+	+	+	+	+	+	+
	<i>P. syringae</i>	-	-	+	-	-	+	-	-	+	-	-	+
	<i>E. chrysanthemi</i>	-	-	+	-	-	+	-	-	+	-	-	+
	<i>E. amylovora</i>	-	-	+	-	-	-	-	-	+	-	-	+

40

- = No symptoms,  
+ = Necrosis with yellow halo, typical of wildfire disease  
+ + = Severe necrosis with yellow halo, typical of wildfire disease

45

The results indicate that the harpin preparations from the three bacteria are effective in

- 41 -

inducing resistance to the wildfire pathogen. Plants treated with either harpin exhibited no symptoms with the two lower inoculum concentrations used. At the higher concentration, symptoms were more severe on buffer-  
5 treated plants than harpin-treated plants.

**Example 9 - Harpin induced resistance against the Late Blight disease caused by *Phytophthora infestans*.**

10

The late blight pathogen affects potatoes and tomatoes primarily. It was responsible for the infamous Irish potato famine. The activity of harpin in inducing resistance to this pathogen was tested on tomato  
15 seedlings grown in the greenhouse. Three-week old seedlings (cultivar 'Mama Mia', about 6 to 8 inches high) were treated with harpin and subsequently inoculated with *Phytophthora infestans*. Two panels of a lower leaf of each plant were infiltrated with a solution of harpin, a  
20 suspension of *Escherichia coli* DH5(pCPP430), which produces and secretes harpin, or potassium phosphate buffer.

Two, three, or four days following infiltration, the plants were inoculated with a mycelial  
25 suspension of *Phytophthora infestans*. The strain U.S. 7 was used, which is highly virulent to tomato. The mycelial suspension was made by blending gently the contents of two barley-meal agar plates on and in which the fungus had grown for 2 weeks at 21°C. The suspension  
30 was brushed onto the top and undersides of one leaf per treated plant with an artist's broad paint brush.

The treated and inoculated plants were incubated in a specially constructed mist chamber designed to maintain a temperature of 20-23°C in the  
35 greenhouse, while maintaining high relative humidity. The moisture was provided by several cool-mist foggers operating at maximum rate on purified water. Disease incidence was evaluated 13 days following inoculation with *Phytophthora infestans*, and the results are

- 42 -

tabulated in Table 9. Each treatment was applied to four individual plants.

Table 9. Numbers of lesion of late blight that were present on tomato leaves 13 days after inoculation.

<u>Treatment</u>		<u>Days between treatment and inoculation</u>											
10	Plant	<u>4</u>				<u>3</u>				<u>2</u>			
		A	B	C	D	A	B	C	D	A	B	C	D
	Buffer	3	2	0	0	1	2	2	0	0	0	4	1
	Harpin	0	0	1	0	0	0	0	1	2	1	0	0
	DH5 (pCPP430)	0	0	0	1	0	2	2	1	0	1	1	0

15

Treatment with harpin reduced the number of lesions that developed on plants at all intervals between treatment and inoculation. The number of late blight lesions that developed also was reduced by prior treatment with DH5(pCPP430), which produces and secretes harpin.

20

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

25

- 43 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: HYPERSENSITIVE RESPONSE  
INDUCED RESISTANCE IN PLANTS
- (iii) NUMBER OF SEQUENCES: 9
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
  - (B) STREET: Clinton Square, P.O. Box 1051
  - (C) CITY: Rochester
  - (D) STATE: New York
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/475,775
  - (B) FILING DATE: 07-JUN-1995
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Goldman, Michael L.
  - (B) REGISTRATION NUMBER: 30,727
  - (C) REFERENCE/DOCKET NUMBER: 19603/10051
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (716) 263-1304
  - (B) TELEFAX: (716) 263-1600

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 338 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

- 44 -

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
 1 5 10 15  
 Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
 20 25 30  
 Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr  
 35 40 45  
 Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu  
 50 55 60  
 Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser  
 65 70 75 80  
 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
 85 90 95  
 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp  
 100 105 110  
 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln  
 115 120 125  
 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met  
 130 135 140  
 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly  
 145 150 155 160  
 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly  
 165 170 175  
 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu  
 180 185 190  
 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala  
 195 200 205  
 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val  
 210 215 220  
 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
 225 230 235 240  
 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
 245 250 255  
 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
 260 265 270  
 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
 275 280 285  
 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
 290 295 300  
 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
 305 310 315 320  
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
 325 330 335  
 Asn Ala

(2) INFORMATION FOR SEQ ID NO:2:

- 45 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2141 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTGCA	CACCGTTACG	60
GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120
GATCTGGTAT	TTCAGTTTGG	GGACACCGGG	CGTGAACCTCA	TGATGCAGAT	TCAGCCGGGG	180
CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTTGAACT	GGCGGGAATG	360
ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
CGATCATTAA	GATAAAGGCG	GCTTTTTTTT	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480
CACCGTCGGC	GTCACTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGGCGTG	540
GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
TCAGGGACTG	AAAGGACTGA	ATTCCGCGGC	TTCATCGCTG	GGTTCAGCG	TGGATAAACT	720
GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATGTTTG	GCGGCGCGCT	780
GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840
TTTCGGCAAT	GGCGCGCAGG	GTGCGAGCAA	CCTGCTATCC	GTACCGAAAT	CCGGCGGCGA	900
TGCGTTGTCA	AAAATGTTTG	ATAAAGCGCT	GGACGATCTG	CTGGGTCATG	ACACCGTGAC	960
CAAGCTGACT	AACCAGAGCA	ACCAACTGGC	TAATTCAATG	CTGAACGCCA	GCCAGATGAC	1020
CCAGGGTAAT	ATGAATGCGT	TCGGCAGCGG	TGTGAACAAC	GCACTGTCGT	CCATTCTCGG	1080
CAACGGTCTC	GGCCAGTCGA	TGAGTGGCTT	CTCTCAGCCT	TCTCTGGGGG	CAGGCGGCTT	1140
GCAGGGCCTG	AGCGGCGCGG	GTGCATTCAA	CCAGTTGGGT	AATGCCATCG	GCATGGGCGT	1200
GGGGCAGAAT	GCTGCGCTGA	GTGCGTTGAG	TAACGTCAGC	ACCCACGTAG	ACGGTAACAA	1260
CCGCCACTTT	GTAGATAAAG	AAGATCGCGG	CATGGCGAAA	GAGATCGGCC	AGTTTATGGA	1320
TCAGTATCCG	GAAATATTCG	GTAAACCGGA	ATACCAGAAA	GATGGCTGGA	GTTCGCCGAA	1380
GACGGACGAC	AAATCTGGG	CTAAAGCGCT	GAGTAAACCG	GATGATGACG	GTATGACCGG	1440
CGCCAGCATG	GACAAATTCC	GTCAGGCGAT	GGGTATGATC	AAAAGCGCGG	TGGCGGGTGA	1500
TACCGGCAAT	ACCAACCTGA	ACCTGCGTGG	CGCGGGCGGT	GCATCGCTGG	GTATCGATGC	1560
GGCTGTCTGC	GGCGATAAAA	TAGCCAACAT	GTCGCTGGGT	AAGCTGGCCA	ACGCCTGATA	1620

- 46 -

ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680  
 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740  
 ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800  
 GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860  
 CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTCTATCC GCCCCTTTAG 1920  
 CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980  
 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040  
 AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100  
 GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 385 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15  
 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30  
 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45  
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60  
 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175

- 47 -

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365  
 Gly Asn Leu Gln His Ala Val Pro Val Val Leu Arg Trp Val Leu Met  
 370 375 380  
 Pro  
 385

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1158 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATGAGTCTGA ATACAAGTGG GCTGGGAGCG TCAACGATGC AAATTTCTAT CGGCGGTGCC	60
GGCGGAAATA ACGGGTTGCT GGGTACCACT CGCCAGAATG CTGGGTGGG TGGCAATTCT	120
GCACTGGGGC TGGGCGGCGG TAATCAAAAT GATACCGTCA ATCAGCTGGC TGGCTTACTC	180
ACCGGCATGA TGATGATGAT GAGCATGATG GCGGTGGTG GGCTGATGGG CCGTGGCTTA	240
GGCGGTGGCT TAGGTAATGG CTTGGGTGGC TCAGGTGGCC TGGGCGAAGG ACTGTGGAAC	300



- 48 -

```

GCGCTGAACG ATATGTTAGG CGGTTGCTG AACACGCTGG GCTCGAAAGG CGGCAACAAT      360
ACCACTTCAA CAACAAATTC CCCGCTGGAC CAGGCGCTGG GTATTAATC AACGTCCCAA      420
AACGACGATT CCACCTCCGG CACAGATTCC ACCTCAGACT CCAGCGACCC GATGCAGCAG      480
CTGCTGAAGA TGTTAGCGA GATAATGCAA AGCCTGTTTG GTGATGGGCA AGATGGCACC      540
CAGGGCAGTT CCTCTGGGGG CAAGCAGCCG ACCGAAGGCG AGCAGAACGC CTATAAAAAA      600
GGAGTCACTG ATGCGCTGTC GGGCCTGATG GGTAATGGTC TGAGCCAGCT CCTTGGCAAC      660
GGGGGACTGG GAGGTGGTCA GGGCGGTAAT GCTGGCACGG GTCTTGACGG TTCGTCGCTG      720
GGCGGCAAAG GGCTGCAAAA CCTGAGCGGG CCGGTGGACT ACCAGCAGTT AGGTAACGCC      780
GTGGGTACCG GTATCGGTAT GAAAGCGGGC ATTCAGGCGC TGAATGATAT CGGTACGCAC      840
AGGCACAGTT CAACCCGTTT TTTCGTCAAT AAAGGCGATC GGGCGATGGC GAAGGAAATC      900
GGTCAGTTCA TGGACCAGTA TCCTGAGGTG TTTGGCAAGC CGCAGTACCA GAAAGGCCCCG      960
GGTCAGGAGG TGAAAACCGA TGACAAATCA TGGGCAAAG CACTGAGCAA GCCAGATGAC     1020
GACGGAATGA CACCAGCCAG TATGGAGCAG TTCAACAAAG CCAAGGGCAT GATCAAAAGG     1080
CCCATGGCGG GTGATACCGG CAACGGCAAC CTGCAGCACG CGGTGCCGGT GGTTCTTCGC     1140
TGGGTATTGA TGCCATGA                                     1158

```

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 341 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## .(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met  Gln  Ser  Leu  Ser  Leu  Asn  Ser  Ser  Ser  Leu  Gln  Thr  Pro  Ala  Met
1      5      10      15
Ala  Leu  Val  Leu  Val  Arg  Pro  Glu  Ala  Glu  Thr  Thr  Gly  Ser  Thr  Ser
20     25     30
Ser  Lys  Ala  Leu  Gln  Glu  Val  Val  Val  Lys  Leu  Ala  Glu  Glu  Leu  Met
35     40     45
Arg  Asn  Gly  Gln  Leu  Asp  Asp  Ser  Ser  Pro  Leu  Gly  Lys  Leu  Leu  Ala
50     55     60
Lys  Ser  Met  Ala  Ala  Asp  Gly  Lys  Ala  Gly  Gly  Gly  Ile  Glu  Asp  Val
65     70     75     80
Ile  Ala  Ala  Leu  Asp  Lys  Leu  Ile  His  Glu  Lys  Leu  Gly  Asp  Asn  Phe
85     90     95
Gly  Ala  Ser  Ala  Asp  Ser  Ala  Ser  Gly  Thr  Gly  Gln  Gln  Asp  Leu  Met
100    105    110

```

- 49 -

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160  
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175  
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190  
 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205  
 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220  
 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240  
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255  
 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270  
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335  
 Asn Gln Ala Ala Ala  
 340

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1026 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTTG	60
GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC	120
GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180

- 50 -

```

AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC      240
ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG      300
GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC      360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC      420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC      480
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC      540
GAAACGGCTG CGTTCCGTTT GGCACCTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG      600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC      660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC      720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA      780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCGCGCAGAC CGGTACGTCG      840
GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG      900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT      960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA     1020
GCCTGA                                           1026

```

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 344 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1           5           10           15
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20           25           30
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
35           40           45
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
50           55           60
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65           70           75           80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
85           90           95
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
100          105          110

```

- 51 -

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
 115 120 125  
 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
 130 135 140  
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160  
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175  
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190  
 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205  
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 Gln Ser Thr Ser Thr Gln Pro Met  
 340

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1035 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60  
 AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120  
 GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180

- 52 -

```

GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC      240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC      300
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA      360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG      420
GGCAACGGCG TGGCGGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC      480
GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC      540
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT      600
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC      660
GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC      720
CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG      780
ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGCGGGCTC GAAGGGTGCC      840
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT      900
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC      960
GTCCAGATCC TGACGAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG     1020
ACGCAGCCGA TGTAAT                                     1035

```

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1       5              10              15
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
                20              25

```

## WHAT IS CLAIMED:

1. A method of imparting pathogen resistance to plants comprising:

5           applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant under conditions where the polypeptide or protein contacts cells of the plant.

10           2. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia amylovora*, *Erwinia chrysanthemi*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas*  
15 *campestris*, and mixtures thereof.

            3. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.  
20

            4. A method according to claim 3, wherein the hypersensitive response elicitor polypeptide or protein has an amino acid sequence corresponding to SEQ. ID. No. 1.

25           5. A method according to claim 3, wherein the hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa.

30           6. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

            7. A method according to claim 6, wherein the hypersensitive response elicitor polypeptide or protein has  
35 an amino acid sequence corresponding to SEQ. ID. No. 3.

8. A method according to claim 6, wherein the hypersensitive response elicitor polypeptide or protein has a molecular weight of 37 kDa.

5           9. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

10           10. A method according to claim 9, wherein the hypersensitive response elicitor polypeptide or protein has an amino acid sequence corresponding to SEQ. ID. No. 5.

15           11. A method according to claim 9, wherein the hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa.

20           12. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

            13. A method according to claim 12, wherein the hypersensitive response elicitor polypeptide or protein has an amino acid sequence corresponding to SEQ. ID. No. 7.

25           14. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

30           15. A method according to claim 14, wherein the hypersensitive response elicitor polypeptide or protein has an amino acid sequence corresponding to SEQ. ID. No. 9.

35           16. A method according to claim 1, wherein the plant is selected from the group consisting of dicots and monocots.

            17. A method according to claim 16, wherein the plant is selected from the group consisting of rice, wheat,

- 55 -

barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

18. A method according to claim 16, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

19. A method according to claim 1, wherein the pathogen to which the plant is resistant is selected from the group consisting of a viruses, bacteria, fungi, and combinations thereof.

20. A method according to claim 1, wherein said applying is carried out by spraying, injection, or leaf abrasion at a time proximate to when said applying takes place.

21. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied to plants as a composition further comprising a carrier.

22. A method according to claim 21, wherein the carrier is selected from the group consisting of water and aqueous solutions.

23. A method according to claim 21, wherein the composition contains greater than 500 nM of the hypersensitive response elicitor polypeptide or protein.

24. A method according to claim 21, wherein the composition further contains additives selected from the



group consisting of fertilizer, insecticide, fungicide, and mixtures thereof.

25. A method according to claim 1, wherein the  
5 hypersensitive response elicitor polypeptide or protein is in isolated form.

26. A method according to claim 1, wherein the  
10 hypersensitive response elicitor polypeptide or protein is applied as bacteria which do not cause disease and are transformed with a gene encoding the hypersensitive response elicitor polypeptide or protein.

27. A method according to claim 1, wherein the  
15 hypersensitive response elicitor polypeptide or protein is applied as bacteria which cause disease in some plant species, but not in those subjected to said applying, and contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

20 28. A method according to claim 1, wherein said applying causes infiltration of the polypeptide or protein into the plant.

25 29. A pathogen-resistant plant with cells in contact with non-infectious hypersensitive response elicitor polypeptide or protein.

30 30. A pathogen-resistant plant according to claim 29, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia amylovora*, *Erwinia chrysanthemi*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof.

35 31. A pathogen-resistant plant according to claim 30, wherein the hypersensitive response elicitor polypeptide

- 57 -

or protein corresponds to that derived from *Erwinia chrysanthemi*.

32. A pathogen-resistant plant according to claim  
5 31, wherein the hypersensitive response elicitor polypeptide  
or protein has an amino acid sequence corresponding to SEQ.  
ID. No. 1.

33. A pathogen-resistant plant according to claim  
10 30, wherein the hypersensitive response elicitor polypeptide  
or protein corresponds to that derived from *Erwinia amylovora*.

34. A pathogen-resistant plant according to claim  
15 33, wherein the hypersensitive response elicitor polypeptide  
or protein has an amino acid sequence corresponding to SEQ.  
ID. No. 3.

35. A pathogen-resistant plant according to claim  
20 30, wherein the hypersensitive response elicitor polypeptide  
or protein corresponds to that derived from *Pseudomonas syringae*.

36. A pathogen-resistant plant according to claim  
25 35, wherein the hypersensitive response elicitor polypeptide  
or protein has an amino acid sequence corresponding to SEQ.  
ID. No. 5.

37. A pathogen-resistant plant according to claim  
30 30, wherein the hypersensitive response elicitor polypeptide  
or protein corresponds to that derived from *Pseudomonas solanacearum*.

38. A pathogen-resistant plant according to claim  
35 37, wherein the hypersensitive response elicitor polypeptide  
or protein has an amino acid sequence corresponding to SEQ.  
ID. No. 7.

39. A pathogen-resistant plant according to claim 30, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

5

40. A pathogen-resistant plant according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein has an amino acid sequence corresponding to SEQ. ID. No. 9.

10

41. A pathogen-resistant plant according to claim 29, wherein the plant is selected from the group consisting of dicots and monocots.

15

42. A pathogen-resistant plant according to claim 41, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

25

43. A pathogen-resistant plant according to claim 41, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

30

44. A pathogen-resistant plant according to claim 30, wherein the pathogen to which the plant is resistant is selected from the group consisting of a virus, bacterium, fungus, and combinations thereof.

35

45. A pathogen-resistant plant according to claim 29, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.

- 59 -

46. A pathogen-resistant plant according to claim 29, wherein the plant cells are in contact with bacteria which do not cause disease and are transformed with a gene encoding the hypersensitive response elicitor polypeptide or protein.

47. A pathogen-resistant plant according to claim 29, wherein the plant cells are in contact with bacteria which do not cause disease in the plant, but do cause disease in other plant species, and contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

48. A pathogen-resistant plant according to claim 29, wherein the plant is infiltrated with the polypeptide or protein.

49. A composition for imparting pathogen resistance to plants comprising:  
a non-infectious hypersensitive response elicitor polypeptide or protein and  
a carrier.

50. A composition according to claim 49, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia amylovora*, *Erwinia chrysanthemi*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof.

51. A composition according to claim 50, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

52. A composition according to claim 50, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

- 60 -

53. A composition according to claim 50, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

5 54. A composition according to claim 50, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

10 55. A composition according to claim 50, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

15 56. A composition according to claim 49, wherein the carrier is selected from the group consisting of water and aqueous solutions.

20 57. A composition according to claim 49, wherein the composition contains greater than 500 nM of the hypersensitive response elicitor polypeptide or protein.

25 58. A composition according to claim 49, wherein the composition further contains additives selected from the group consisting of fertilizer, insecticide, fungicide, and mixtures thereof.

59. A composition according to claim 49, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.

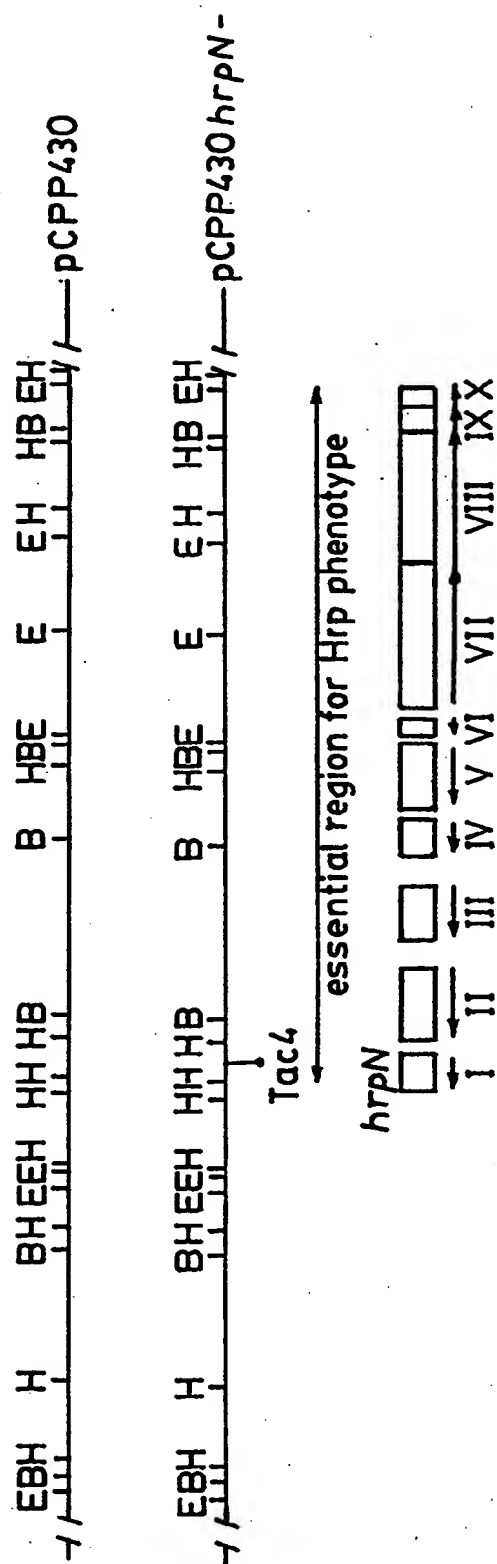
30 60. A composition according to claim 49, wherein the hypersensitive response elicitor polypeptide and protein is produced or capable of being produced by bacteria in the composition, said bacteria do not cause disease and are transformed with a gene encoding the hypersensitive response  
35 elicitor polypeptide or protein.

61. A composition according to claim 49, wherein the hypersensitive response polypeptide or protein is

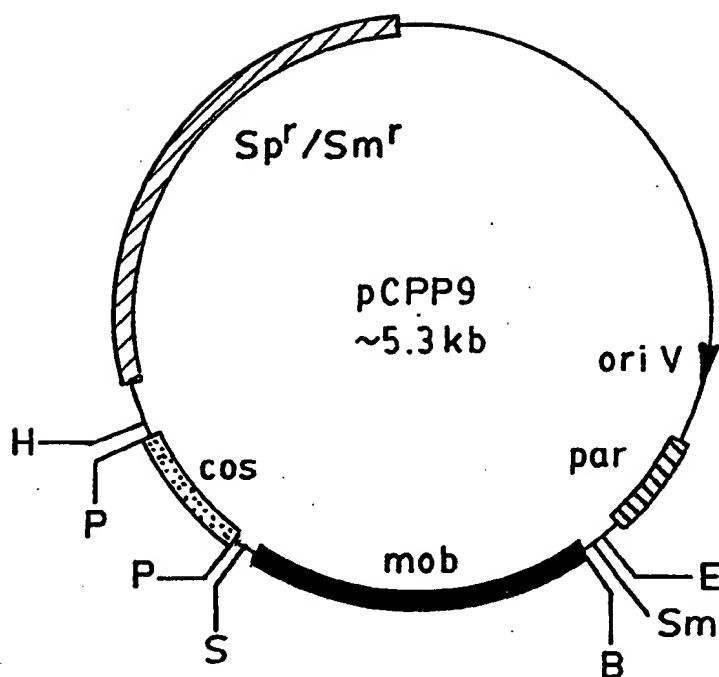
- 61 -

produced or capable of being produced by bacteria capable of causing disease in plants and containing a gene encoding the hypersensitive response elicitor polypeptide or protein.

1/2

FIG. 1

2/2

FIG. 2



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08819

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01G 13/00; A61K 35/66; C12N 1/20, 9/00; C12R 1:18  
US CL :47/58; 435/252.1, 847; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 47/58; 435/252.1, 847; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOCCARA et al. 'Plant Defense Elicitor Protein Produced by <i>Erwinia chrysanthemi</i> .' In: Mechanisms of Plant Defense Responses. Edited by B. Fritig et al. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1993, page 166, see entire document.	1-5, 16-25, 28-32, 41-45, 48-51, 56-59
Y	WEI et al. Hrpl of <i>Erwinia amylovora</i> Functions in Secretion of Harpin and Is a Member of a New Protein Family. Journal of Bacteriology. December 1993, Vol. 175, No. 24, pages 7958-7967, especially pages 7958-7959.	1-5, 16-25, 28-32, 41-45, 48-51, 56-59

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

16 AUGUST 1996

Date of mailing of the international search report

26 AUG 1996

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DAVID T. FOX

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08819

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BAUER et al. <i>Erwinia chrysanthemi hrp</i> Genes and Their Involvement in Soft Rot Pathogenesis and Elicitation of the Hypersensitive Response. MPMI. 1994, Vol. 7, No. 5, pages 573-581, especially pages 573, 574 and 578.	1-5, 16-25, 28-32, 41-45, 48-51, 56-59

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US96/08819

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-5, 16-25, 28-32, 41-45, 48-51, 56-59

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08819

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

- I. Claims 3-5, 25, 31-32, 45, 51 and 59, drawn to methods of topically applying an *Erwinia chrysanthemi* protein to a plant, classified in Class 514, subclass 2, for example.
- II. Claims 6-8, 25, 33-34, 45, 52 and 59, drawn to methods of topically applying an *Erwinia amylovora* protein to a plant, classified in Class 514, subclass 2, for example.
- III. Claims 9-11, 25, 35-36, 45, 53 and 59, drawn to methods of topically applying a *Pseudomonas syringae* protein to a plant, classified in Class 514, subclass 2, for example.
- IV. Claims 12-13, 25, 37-38, 45, 54 and 59, drawn to methods of topically applying a *Pseudomonas solanacearum* protein to a plant, classified in Class 514, subclass 2, for example.
- V. Claims 14-15, 25, 39-40, 45, 55 and 59, drawn to methods of topically applying a *Xanthomonas campestris* protein to a plant, classified in Class 514, subclass 2, for example.
- VI. Claims 3-5, 26-27, 31-32, 46-47, 51 and 60-61, drawn to methods of applying bacteria transformed with a gene encoding an *Erwinia chrysanthemi* protein to a plant, classified in Class 424, subclass 93.2, for example.
- VII. Claims 6-8, 26-27, 33-34, 46-47, 52 and 60-61, drawn to methods of applying bacteria transformed with a gene encoding an *Erwinia amylovora* protein to a plant, classified in Class 424, subclass 93.2, for example.
- VIII. Claims 9-11, 26-27, 35-36, 46-47, 53 and 60-61, drawn to methods of applying bacteria transformed with a gene encoding a *Pseudomonas syringae* protein to a plant, classified in Class 424, subclass 93.2, for example.
- IX. Claims 12-13, 26-27, 37-38, 46-47, 54 and 60-61, drawn to methods of applying bacteria transformed with a gene encoding a *Pseudomonas solanacearum* protein to a plant, classified in Class 424, subclass 93.2, for example.
- X. Claims 14-15, 26-27, 39-40, 46-47, 55 and 60-61, drawn to methods of applying bacteria transformed with a gene encoding a *Xanthomonas campestris* protein to a plant, classified in Class 424, subclass 93.2, for example.

Claims 1-2, 16-24, 28-30, 41-44, 48-50 and 56-58 are generic.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The claims are not drawn to a single protein or a single gene encoding that protein. Instead, claims 1-2, 16-30, 41-50 and 56-61 are generically drawn to a multitude of biochemically divergent proteins which have a multitude of biochemically divergent sequences, and which are from divergent microbial sources.

Furthermore, the claims are not drawn to a single method of protecting plants from disease. Instead, claims 1-24, 28-44 and 48-58 are generically drawn to any method of protecting plants from disease, which method could include the topical application of an isolated protein, or the application of a bacterium which has been transformed with a gene encoding that protein. The protein is physiologically and biochemically distinct from a gene or bacterium, and the methods for obtaining and applying each would not be required by the other.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.